

TITLE: ANALYSIS OF AMINOSUGARS IN TOBACCO BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORIMETRIC DETECTION

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ABSTRACT: A liquid chromatographic method for the quantitative determination of glucosamine, galactosamine, and mannosamine in flue-cured and burley tobaccos in the range of 2.0-0.01% was developed. This method consists of the extraction of one gram of tobacco with water, adjusted to pH 7 using sodium phosphate. The extract is then filtered and placed in an autosampler vial. Immediately before injection the sample is derivatized with o-phthalaldehyde (OPA) inside a Waters® autosampler. Separation is performed in a Waters® RCSS® C-18 column, 5 μ m, with an 85% water, 15% tetrahydrofuran mobile phase adjusted to pH 7 with sodium phosphate, at a flowrate of 2.0 ml/min. Recovery of the three aminosugars was not statistically different from 100%. The relative standard deviation (RSD) for six different extractions was 5.5% for each compound analyzed. The RSD for 10 injections of the same sample was 10% for mannosamine and 6% for glucosamine and galactosamine. Excitation and emission wavelengths used for this analysis were 229 nm and 458 nm, respectively. At these wavelengths no coelution was observed. Using another mobile phase and stopped-flow fluorescence spectrophotometry, as well as wavelength ratioing of standards and samples, the specificity of the separation and detection were verified.

REVIEW: The main purpose of this work was the measurement of tobacco-identical flavor precursors in various grades of bright and burley tobaccos. A rapid liquid chromatographic technique was reported which allows the routine quantitation of the tobacco-identical glucosamine and mannosamine. Galactosamine was not found in the tobacco samples utilized in this presentation, but it was included as a component of interest for the method development. The instrumental technique as reported is automated for high sample output and routine sample analysis. Details of the relative standard deviation and recovery study statistics with the above three aminosugars were reported.

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